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Epidemiological Investigation and Antibiotic Sensitivity of Salmonellosis in Goats at the Selected Areas of Bangladesh

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ABSTRACT

Salmonellosis is one of the life-threating diseases of goat in Bangladesh. Therefore, the present study was designed to study the prevalence of Salmonellosis, and isolation and characterizations of the *Salmonella* spp. from apparently healthy and diarrheic goat. A total of 47 faces samples were collected from selected place and cultured onto different prescribed medium to isolate it. In this study, 12.76% (6/47) samples were found to be positive for *Salmonella* spp. During culture on SS agar medium, all of the *Salmonella* isolates produced round, smooth, opaque, translucent and black color colonies on SS agar media. All of the isolated *Salmonella* spp. fermented dextrose, maltose and mannitol with production of acid and gas but did not ferment sucrose and lactose. However, these isolates had showed Indole and Voges-Proskauer test negative, Methyl-Red test positive. All of these isolates were subjected to rapid plate agglutination test with polyvalent "O" (Poly 'O') and polyvalent "H" (poly 'H') antisera where positive agglutination were observed. They were highly sensitive to ciprofloxacin, spiramycin and gentamycin; moderately sensitive to oxytetracyline, streptomycin and amoxicillin; less sensitive to sulphamethoxazole and resistant to penicillin-G. Based on the present findings, it may be concluded that the investigated *Salmonella* spp. from goats might be *S. typhimurium, S. enteritidis, S. brandenburg, S. salford, S. newbrunswick, S. newport* or *S. dublin.* Further study will be needed, therefore it requires further characterization using other serological and molecular techniques.

(Key words: Bangladesh, goat, salmonellosis, prevalence, sensitivity)

INTRODUCTION

Salmonellosis is a disease or a group of diseases caused by a wide variety of *Salmonella* serovars in various hosts including goat (OIE Manual, 2006) which remain a serious problem with public health significance throughout the world (Tabaraie *et al*., 1994). Substantial economic loss results from mortality and poor growth of animals, birds and human beings as well as the hazards of transmitting food poisoning with gastroenteritis to human and representing a serious problem for the food industry (Cooke and Todd, 1990). The goat population of Bangladesh is suffering from a contagious disease such as Brucellosis, Salmonellosis, Enterotoxaemia, *Peste des petits* ruminants, Goat pox and contagious ecthyma. From the reports of personnel communication of the field cases of various corners of Bangladesh, it is assumed that Salmonellosis is one of the most prevalence among them. *Salmonellae* are Gram negative, small rod shaped, nonsporeforming, noncapsulated, aerobic and facultatively anaerobic organisms classified under the family Enterobacteriaceae (Freeman, 1985; Gene, 2002; OIE Manual, 2006). Salmonellosis assumes to occur as one of the following forms: peracute septicemia, acute enteritis, chronic enteritis or a subclinical carrier state. Common clinical signs are septicemia, fever, enteritis, arthritis and also abortion. But the clinical signs may vary from species to species (Blood *et al*., 2003). *Salmonella* infection in goat occurs in all ages, all seasons both in male and female and is responsible for a considerable loss of kids and even may cause abortion in adults (Arruda *et al*., 2004). This infection may be a problem in chevon raising and goat rearing industry and in areas where this will surely impair

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the fattiness and sound health of goat. The poor quality and quantity of chevon leads to poor market value (Arruda *et al*., 2004). A mortality rate of 14.52% in kids was reported in India due to Salmonellosis (Ghosh *et al*., 1987).

Salmonella organisms were isolated from poultry (Begum, 1992), cattle (Islam, 2007), sheep (Kobayashi, 2007), ruminants (Rahman, 2007). However, the epidemiological investigation of Salmonellosis in goat has not been studied in Bangladesh. The goat farmers also used of antibiotics to treat infections indiscriminately without confirmatory diagnosis. As a result, drug resistant *Salmonella* organisms are being emerged. In view of the above consideration, the present study was undertaken a) to study the prevalence of Salmonellosis in goat of some selected areas of Bangladesh considering age, sex, breed, seasons and health status. b) To isolate and identify the *Salmonella* spp. from clinical specimens of goat. c) To characterize the *Salmonella* of goat isolates using cultural, biochemical and serological techniques. d) To develop remedial measures against the isolated *Salmonella* spp.

MATERIALS AND METHODS

1. Selected Area for Study

Samples were collected of Bangladesh i.e. Bangladesh Agricultural University Veterinary clinic, goat farm, Babul goat farm, Aziz coloni, Boira, Mymensingh and Savar goat farm. A total number of 47 faecal samples were collected from apparently healthy and diarrheic goats and then transferred.

2. Culture of *Salmonella* spp.

Nutrient broth was used to grow the *Salmonella* spp. and performing hanging drop test, biochemical test and antibiotic sensitivity test (Cheesebrough, 1984). Nutrient agar medium was used to grow the *Salmonella* spp. from the collected samples (Cheesebrough, 1984). Salmonella-shigella (SS) agar medium was used as a selective medium for *Salmonella* spp. which causes enhancement of the growth of *Salmonella* while inhibiting the growth of other contaminating organisms and shows typical colony characters (Cheesebrough, 1984). Brilliant green agar (BGA) medium was used as a selected medium for the isolation of *Salmonella* spp. (Cheesebrough, 1984). MacConkey (MC) agar medium was used for culturing the organisms under the family Enterobacteriaceae (Cheesebrough, 1984). Eosin methylene blue (EMB) agar medium was used for the purpose of observing differential growth of *Salmonella* spp. and other enterobacters specially *Escherichia* coli (Cheesebrough, 1984). Triple sugar iron (TSI) agar slant was used for the purpose of observing characteristics colony of the isolated *Salmonella* spp. and for the preservation of bacteria. Blood agar medium was used to perform the antibiotic sensitivity study.

3. Morphological Characterization by Gram's Method

The representative *Salmonella* colonies were characterized morphologically using Gram's staining technique according to the method described by Merchant and Packer (1967).

4. Identification of Suspected *Salmonella* Isolates using Biochemical test

1) Sugar Fermentation Test

The carbohydrate fermentation test was performed by inoculating 0.2 ml of nutrient broth culture of the isolated organisms into the tubes containing different sugar media (five basic sugars such as dextrose, maltose, lactose, sucrose and mannitol) and incubated for 24 hours at 37℃. Acid production was indicated by the color change from pink to yellow and gas production was noted by the accumulation of gas bubbles in the inverted Durham's tube (Cheesbrough, 1985).

2) Methyl Red Test

The test was conducted by inoculating single colony from the pure culture of the test organism in 5 ml sterile MR broth. After 5 days incubation at 37℃, 5 drops of methyl red solution was added and observed for color formation. Development of red color was positive and indicated an acid pH of 4.5～6 resulting from the fermentation of glucose. Development of yellow color indicated negative results (Cheesbrough, 1985).

3) Voges-Proskauer Test

Two ml of sterile glucose phosphate peptone water were inoculated with the 5 ml of test organisms. It was incubated at 37℃ aerobically for 48 hours. A very small amount (knife-point) of creatine was added and mixed. 3 ml of sodium hydroxide were added and shake well. The bottle cap was removed and left for an hour at the room temperature. It was observed closely for the slow development of pink color positive cases. In negative cases, there was no development of pink color (Cheesbrough, 1985).

4) Indole Test

The test organisms were cultured in test tubes having 5 ml

of peptone water containing tryptophan at 37℃ for 48 hours. Then 1 ml of diethyl ether was added, shakes well and allowed to stand until the ether rises to the top. Then 0.5 ml of Kovac's reagent was gently run down the side of the test tube so that it forms a ring in between the medium and the ether layer and observed for the development of color of the ring. Development of a brilliant red colored ring indicated indole production. In negative case there is no development of red color (Cheesbrough, 1985).

5) Serotyping through Slide Agglutination Test

Salmonella agglutinating antiserum poly "O" and poly "H" (S & E reagents Lab, Bangkok, Thailand) was used to perform the serotyping of the isolated *Salmonella* spp. The macroscopic slide agglutination tests were performed. The cultures to be tested were first checked with *Salmonella* "poly-O" polyvalent antiserum. A single isolated colony from SS agar was emulsified with physiological saline solution. A single drop of thick bacterial suspension was placed on glass slide and a drop of polyvalent antiserum was added. The slide was gently rotated to mix the fluid thoroughly. These cultures which agglutinated within one to two minutes were selected as positive for *Salmonella* and subjected to agglutination test with *Salmonella* agglutinating antiserum (poly "H"). According to manufacturer's direction, it was noted that poly "O" antiserum gives positive agglutination reaction with any serovars for preliminary screening of *Salmonella* and poly "H" antiserum gives specific agglutination reaction for motile *Salmonella* spp. (Buxton and Fraser, 1977).

6) Antibiogram Study of the Isolated *Salmonella* spp.

Susceptibility of the isolated *Salmonella* to different antibacterial agents were performed through disc diffusion method to determine the drug sensitivity pattern according to the method described by Bauser *et al*. (1966).

RESULTS AND DISCUSSION

1. Isolation of *Salmonella* spp. from Feces Samples

The results of isolation of *Salmonella* from different faeces samples were shown in Table 1. Out of 47 samples examined six were found to be positive for *Salmonella*. Among the positive samples, three were from BAU Veterinary clinics, two were from BAU goat farm and one was from Savar goat farm. The prevalence rate of *Salmonella* in goat of these study areas were 25%, 15.38%, 10% respectively. In Babul goat farm no sample were found to be positive for *Salmonella*.

2. Prevalence of *Salmonella* Infection

The prevalence of Salmonellosis was found to be higher in young (16.67%), sick (44.44%), local breed (16%) with equal susceptibility of both male (50%) and female (50%) goat, which was similar to the findings of Mudit *et al*., (2007). The prevalence of Salmonellosis was found to be higher in summers (19.04%), which also satisfy the findings of Mahendra *et al*., (2006). On the other hand, the prevalence of Salmonellosis is lower in adults, Black Bengal breed goats with good body conditions. *Salmonella* infection in goat occurs in all ages, all season both in male and female and is responsible for considerable loss in kids, even may cause abortion ranging from 22% to 38% during the last-third of gestation in adults (Habrun *et al*., 2006). This infection may be a problem in goat rearing industry and area where this will surely impair the fattiness and sound health of goats and give the poor quality and quantity of goat meat which leads to poor market value (Arruda *et al*., 2004). Infection by *Salmonella* is a common cause of food poisoning in humans (Hobbs and Robert, 1993). The prevalence rate of *Salmonella* organism in the goat of this country is not insignificant and should not be overlooked, because of their public health significance and the possibility of dissemination of diseases in man, animals and birds.

Table 1. Results of isolation of *Salmonella* from faeces samples of apparently healthy and diarrheic goats

SL No.	Study areas	No. of collected sample	No. of positive sample	Prevalence rate (%)	Overall prevalence rate (%)
1	BAU veterinary clinic	12	3	25	12.76 (6/47)
2	BAU goat farm	13	2	15.38	
3	Babul goat farm	12	0	0.0	
4	Savar goat farm	10	1	10	

Table 2. Results showing isolation of *Salmonella* and their prevalence rate in different epidemiological parameters

Epidemiological parameters	Level of patterns	No. of animal examined	No. of animal affected	Prevalence rate (%)
Age	1~12 month	12	2	16.67
	13~24 month	20	3	15
	25~36 month	15	1	6.67
Sex	Male	19	3	15.78
	Female	28	3	10.71
Breed	Local	25	4	16
	Black Bengal	22	2	9.09
Seasons	Rainy	14	1	7.14
	Winter	12	1	8.33
	Summer	21	4	19.04
Health status	Apparently healthy	38	2	5.26
	Sick	9	4	44.44

3. Isolation and Identification of *Salmonella*

Specific enrichment media and biochemical tests were used for the isolation and identification of *Salmonella* which was previously suggested by a number of researchers (Ruiz *et al*., 1992; Dhruba *et al*., 1999; Habrun *et al*., 2006). In this study, colony characteristics of *Salmonella* from different species of animals and birds on MC agar, SS agar and BG agar were similar to the findings of other authors (Shaffer *et al*., 1964; Merchant and Packer, 1967).

In Gram's staining, the morphological characteristics of the isolated *Salmonella* exhibited Gram negative, small rod shaped, single or paired in arrangement under microscope which was supported by other researchers (Freeman, 1985; Jones *et al*., 1997; Gene, 2002). In motility test, all of the isolates of goat were found to be motile. This result was correlated with the results of previous reports (Merchant and Packer, 1967; Buxton and Fraser 1977).

Differentiation of *Salmonella* into species level was difficult based on their sugar fermentation pattern (Freeman, 1985). In sugar fermentation test, all of the isolated *Salmonella* fermented dextrose, maltose and mannitol and produced acid and gas but did not ferment sucrose and lactose which satisfy the statement of Buxton and Fraser (1977). Again all of the isolated *Salmonella* were positives to MR test and negative to indole test and V-P test.

In this study slide agglutination test was performed with commercially available agglutinating polyvalent antisera which is very simple and sensitive (Avakian *et al*., 1988) whereas ELISA test is very much expensive and time consuming (Begum, 2005). All the isolates were agglutinated with both poly "O" and poly "H" antisera which indicated that the isolates were *Salmonella* spp.

4. Results of Antibiogram of the Isolated *Salmonella*

Isolated *Salmonella* spp. showed various degree of sensitivity to oxytetracycline, gentamycin, sulphamethoxazole, spiramycin, streptomycin, amoxicillin, penicillin-G, ciprofloxacin. Out of 6 *Salmonella* isolates from goats, 83.33% were highly sensitive and 16.67% were moderately sensitive to ciprofloxacin and spiramycin respectively; 66.67% were highly sensitive and 33.33% were moderately sensitive to gentamycin; 66.67% were moderately sensitive and 33.33% were less sensitive to oxytetracycline; 66.67% were moderately sensitive and 33.33% were less sensitive to streptomycin; 33.33% were moderately sensitive and 66.67% were less sensitive to amoxicillin; 16.67% were moderately sensitive, 16.67% were less sensitive and 66.66% were resistant to suiphamethoxazole and 16.67% were less sensitive and 83.33% were resistant to penicillin-G. (Table 3). The antibiotic sensitivity tests were performed by disc diffusion method using 8 different antibiotic discs. Most of the *Salmonellae* isolates were highly sensitive to ciprofloxacin, spiramycin and gentamicin; moderately sensitive to oxytetracy-

Table 3. Antibiotic sensitivity pattern of *Salmonella* isolates from goat

Source of sample	Isolated *Salmonella*	Highly sensitive (+++)	Moderately sensitive (++)	Less sensitive (+)	Resistant (-)
G_1	G_{1a}	CIP, SP, GN	S, OT	AML	RL, P
	G_{1b}	SP, GN	CIP, OT	AML, S	RL, P
	G_{1c}	CIP, GN	SP, S	OT, AML	RL, P
G_2	G_{2a}	CIP, SP	GN, S, OT	AML, RL	P
	G_{2b}	CIP, SP, GN	S, RL, AML	OT	P
G_3	G_{3a}	CIP, SP	OT, AML, GN	P, S	RL

G_{1a}, G_{1b}, G_{1c} = Isolated *Salmonella* from BAU veterinary clinic, G_{2a}, G_{2b} = Isolated *Salmonella* from BAU goat farm, G = Isolated *Salmonella* from savar goat farm, CIP = Ciprofloxacin, SP = Spiramycin, GN = Gentamycin, S = Streptomycin, OT = Oxytetracycline, AML = Amoxicillin, RL = Sulphamethoxazole, P = Penicillin-G.

line, streptomycin, and amoxicillin; less sensitive to sulphamethoxazole and resistant to penicillin-G. These findings satisfy the result of Yadav *et al*., (2006) and Habrun *et al*., (2006). However, the authors found that *S. berta* was moderately sensitive to penicillin. The antibacterial sensitivity pattern of *Salmonella* isolates recorded in this study might be due to indiscriminate use of those antibacterial agents in field condition. This provided the guideline to the veterinarian for selecting appropriate antibiotics. Data of antibacterial sensitivity pattern indicated that antibiotic resistant *Salmonella* isolates were present in goats which might affect the public health.

CONCLUSIONS

From the findings it may be concluded that *Salmonella* was the important cause of goat Salmonellosis in Bangladesh. Slide agglutination test can be used for the rapid detection of *Salmonella* in field cases. Salmonellosis occurred most frequently in young, sick, local breeds of goat. The prevalence of Salmonellosis is higher in summer. Ciprofloxacin, spiramycin and gentamicin were the best choice of drug among the antibacterials available in the market. Variation of antibacterial sensitivity or resistance pattern was observed in this study.

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